

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
29 March 2001 (29.03.2001)

PCT

(10) International Publication Number
WO 01/21780 A3

- (51) International Patent Classification⁷: **C12N 15/82**, 15/64, 15/65, A01H 5/00 (74) Agent: **HARRISON GODDARD FOOTE**; Tower House, Merriam Way, Leeds LS2 8PA (GB).
- (21) International Application Number: **PCT/GB00/03543** (81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.
- (22) International Filing Date:
15 September 2000 (15.09.2000)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:
9921937.0 17 September 1999 (17.09.1999) GB
- (71) Applicant (*for all designated States except US*): **UNIVERSITY OF LEEDS [GB/GB]**; Leeds LS2 9JT (GB).
- (72) Inventors; and
- (75) Inventors/Applicants (*for US only*): **MEYER, Peter** [DE/GB]; Leeds Institute for Plant Biotechnology and Agriculture (LIBA), Faculty of Biological Sciences, The University of Leeds, Leeds LS2 9JT (GB). **ZUBKO, Elena** [UA/GB]; Leeds Institute for Plant Biotechnology and Agriculture (LIBA), Faculty of Biological Sciences, The University of Leeds, Leeds LS2 9JT (GB).
- (84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).
- Published:
— with international search report
- (88) Date of publication of the international search report:
18 October 2001
- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

(54) Title: **TARGETED GENE REMOVAL**

(57) Abstract: A method of removing a part of a transgene after its integration into a genome comprising flanking the part of the transgene on each side with an attachment P region (attP) of bacteriophage λ and inducing intrachromosomal homologous recombination between the flanking attP regions so that the part of the transgene sandwiched between the attP regions is removed.

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INTERNATIONAL SEARCH REPORT

International Application No

PC 98 00/03543

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N15/82 C12N15/64 C12N15/65 A01H5/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12N A01H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PEREDELCHUK M Y ET AL: "A method for construction of E. coli strains with multiple DNA insertions in the chromosome" GENE: AN INTERNATIONAL JOURNAL ON GENES AND GENOMES, GB, ELSEVIER SCIENCE PUBLISHERS, BARKING, vol. 187, no. 2, 18 March 1997 (1997-03-18), pages 231-238, XP004093285 ISSN: 0378-1119 the whole document	1, 2, 4, 9, 16
X	EP 0 299 552 A (SOLVAY ; CLOVIS MATTON N V (BE)) 18 January 1989 (1989-01-18) the whole document	1, 2, 15, 16
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Further documents are listed in the continuation of box C.

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Patent family members are listed in annex.

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Date of the actual completion of the international search

10 April 2001

Date of mailing of the international search report

26/04/2001

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Maddox, A

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 99 21977 A (LIFE TECHNOLOGIES INC) 6 May 1999 (1999-05-06) figures 10B,11A ---	16
P,X	ZUBKO ELENA ET AL: "Intrachromosomal recombination between attP regions as a tool to remove selectable marker genes from tobacco transgenes." NATURE BIOTECHNOLOGY, vol. 18, no. 4, April 2000 (2000-04), pages 442-445, XP002165150 ISSN: 1087-0156 the whole document ---	1-22
E	WO 01 07572 A (UNIV CALIFORNIA ;US AGRICULTURE (US)) 1 February 2001 (2001-02-01) the whole document ---	1-3,5,9, 12-20
A	YODER J I ET AL: "TRANSFORMATION SYSTEMS FOR GENERATING MARKER-FREE TRANSGENIC PLANTS" BIO/TECHNOLOGY,US,NATURE PUBLISHING CO. NEW YORK, vol. 12, no. 3, 1 March 1994 (1994-03-01), pages 263-267, XP002043802 ISSN: 0733-222X the whole document ---	12-15, 17-22
A	OW D W ET AL: "GENOME MANIPULATION THROUGH SITE-SPECIFIC RECOMBINATION" CRITICAL REVIEWS IN PLANT SCIENCES,US,CRC PRESS, BOCA RATON, FL, vol. 14, no. 3, 1995, pages 239-261, XP000614883 ISSN: 0735-2689 the whole document ---	12-15, 17-22
A	EP 0 911 412 A (JUJO PAPER CO LTD) 28 April 1999 (1999-04-28) the whole document ---	4,12-15, 17-22
A	WO 94 17176 A (PURDUE RESEARCH FOUNDATION) 4 August 1994 (1994-08-04) the whole document ---	12-15, 17-22
A	EP 0 160 571 A (BIOTEKNIKA INTERNATIONAL) 6 November 1985 (1985-11-06) the whole document ---	1-22
A	WO 96 33269 A (EINSTEIN COLL MED ;MEDIMMUNE INC (US); UNIV PITTSBURGH (US)) 24 October 1996 (1996-10-24) the whole document ---	1-22

-/--

INTERNATIONAL SEARCH REPORT

International Application No

PC 00/03543

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	HORIE K ET AL: "A replacement vector used to introduce subtle mutations into mouse genes" GENE,NL,ELSEVIER BIOMEDICAL PRESS. AMSTERDAM, vol. 166, no. 2, 1995, pages 197-204, XP004043073 ISSN: 0378-1119 the whole document ---	1-22
A	WO 91 19796 A (BAYLOR COLLEGE MEDICINE) 26 December 1991 (1991-12-26) the whole document ---	1-22
A	D'ENFERT C: "Selection of multiple disruption events in Aspergillus fumigatus using the orotidine-5'-decarboxylase gene, pyrG, as a unique transformation marker" CURRENT GENETICS,US,NEW YORK, NY, vol. 30, no. 1, June 1996 (1996-06), pages 76-82, XP002107254 ISSN: 0172-8083 the whole document -----	1-22

INTERNATIONAL SEARCH REPORT

for on patent family members

International Application No

PCT/B 00/03543

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
EP 0299552	A	18-01-1989	NL 8701450 A AT 104346 T DE 3889021 D DE 3889021 T DK 338788 A JP 2000461 A US 5364779 A	16-01-1989 15-04-1994 19-05-1994 08-09-1994 08-02-1989 05-01-1990 15-11-1994
WO 9921977	A	06-05-1999	AU 1199599 A CN 1280614 T EP 1025217 A	17-05-1999 17-01-2001 09-08-2000
WO 0107572	A	01-02-2001	NONE	
EP 0911412	A	28-04-1999	AU 728915 B AU 2652097 A CA 2253956 A WO 9742334 A JP 10327860 A	18-01-2001 26-11-1997 13-11-1997 13-11-1997 15-12-1998
WO 9417176	A	04-08-1994	US 5527695 A EP 0686191 A US 5910415 A US 6110736 A US 5744336 A	18-06-1996 13-12-1995 08-06-1999 29-08-2000 28-04-1998
EP 0160571	A	06-11-1985	US 4673640 A AU 4179385 A DK 191085 A FI 851682 A JP 60244291 A US 4900669 A	16-06-1987 05-12-1985 31-10-1985 31-10-1985 04-12-1985 13-02-1990
WO 9633269	A	24-10-1996	US 5736367 A AU 5549496 A	07-04-1998 07-11-1996
WO 9119796	A	26-12-1991	AU 654284 B AU 8182391 A CA 2084774 A EP 0535144 A US 5614396 A	03-11-1994 07-01-1992 13-12-1991 07-04-1993 25-03-1997